REMARKS

Claims 1-8 are pending and under examination in the subject application. Applicants have amended claims 1-3, 5 and 7, and canceled claims 4 and 8. Applicants have amended claims 1-3, 5 and 7 to clarify subject matter and make certain format changes. Therefore, applicants maintain that these amendments raise no issue of new matter. Accordingly, claims 1-3 and 5-7 are pending in the subject application.

Rejection under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 1-8 under 35 U.S.C. §112, first paragraph, as allegedly not enabled for a transgenic nonhuman animal comprising separate DNA sequences encoding hAPP695, hAPP751 and hAPP770, and methods of evaluating using the transgenic nonhuman animal.

Specifically, the Examiner alleges that the specification, while enabling for a transgenic mouse whose genome comprises a DNA sequence comprising a nerve tissue specific promoter operatively linked to a DNA sequence encoding ABAD and a nerve tissue specific promoter operatively linked to an alternatively spliced hAPP mini-gene that encodes hAPP695, hAPP751 and hAPP770, comprising one or more familial Alzheimer's disease mutants, and methods of using the mice to evaluate therapeutic treatments, the specification is not enabling for other transgenic nonhuman animals comprising separate DNA sequences encoding hAPP695, hAPP751 and hAPP770, and methods of

evaluating using the transgenic nonhuman animal.

In response, and without conceding the correctness of the Examiner's rejection, applicants note that claims 4 and 8 have been canceled by this Amendment. Accordingly, the Examiner's rejection of those claims is now moot. Additionally, applicants note that independent claims 1 and 5, and dependent claims 2, 3, 6 and 7, as amended, provide a transgenic *rodent*. Applicants maintain that transgenic rodents are enabled, since the transgenic mice acknowledged by the Examiner as enabled constitute a representative example of transgenic rodents.

The Examiner also rejected claims 1-8 under the enablement portion of 35 U.S.C. §112, first paragraph, for allegedly improperly incorporating a reference.

Specifically, the Examiner alleges that the Hsia reference incorporated by the applicants into the specification is critical to the invention, i.e. the production of transgenic ABAD/hAPP mice.

In response to the Examiner's rejection, but without conceding the correctness thereof, applicants note that claims 4 and 8 have been canceled by this Amendment. Accordingly, the Examiner's rejection of those claims is now moot. Furthermore, applicants note that by this Amendment, they have amended the specification to include the pertinent portion of the Hsia article detailing the production of transgenic hAPP mice.

Applicants assert that the amended claims satisfy the requirements of 35 U.S.C. §112, first paragraph, and respectfully request that the Examiner reconsider and withdraw this rejection.

Rejection under 35 U.S.C. §112, Second Paragraph

The Examiner also rejected claims 1-8 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to point out and distinctly claim the subject matter which applicants regard as the invention.

Specifically, the Examiner alleges that with regard to claims 1 and 5, which recite "... a DNA sequence encoding a mutant human amyloid precursor protein hAPP695, hAPP751 and hAPP770...", it is unclear as to whether the DNA sequence gives rise to all three splice variants in the doubly transgenic mice.

In response, and without conceding the correctness of the Examiner's rejection, applicants note that claims 4 and 8 have been canceled by this Amendment. Accordingly, the Examiner's rejection of those claims are now moot. Furthermore, applicants direct the Examiner's attention to amended claims 1 and 5, which now recite, in part, a "DNA sequence encoding *each of* mutant human amyloid precursor proteins hAPP695, hAPP751, and hAPP770..." (emphasis added). Applicants maintain that the language of these claims is clear.

Additionally, the Examiner rejected claims 1-8 under 35 U.S.C.

§112, second paragraph, because while claims 1 and 5 recite amyloid-beta peptide alcohol dehydrogenase, the art defines ABAD as amyloid-beta peptide binding alcohol dehydrogenase or as amyloid beta binding alcohol dehydrogenase.

In response, and without conceding the correctness of the Examiner's rejection, applicants note that claims 1-5, as amended, recite, in part, "amyloid-beta peptide *binding* alcohol dehydrogenase" (emphasis added).

Accordingly, applicants assert that claims 1-3 and 5-7 satisfy the requirements of 35 U.S.C. §112, second paragraph, and respectfully request that the Examiner reconsider and withdraw his rejection.

Information Disclosure Statement

In accordance with their duty of disclosure under 37 C.F.R. §1.56, applicants would like to direct the Examiner's attention to Hsia et al., "Plaque-Independent Disruption of Neural Circuits in Alzheimer's Disease Mouse Models," Proc. Natl. Acad. Sci. U.S.A., 1999 March 16; 96(6): 3228-3233 listed on the attached Form PTO-1449 **(Exhibit 1)** and attached hereto as **Exhibit 2**.

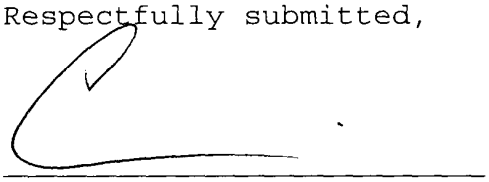
Summary

No fee, other than the $375.00 fee for filing an RCE, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is hereby

given to charge the amount of such fee to Deposit Account No. 03-3125.

If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

Respectfully submitted,



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U.S. PATENT DOCUMENTS

Examiner Initial		Document Number	Date	Name	Class	Subclass	Filing Date if Appropriate

FOREIGN PATENT DOCUMENTS

		Document Number	Date	Country	Class	Subclass	Translation	
							Yes	No

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

		Hsia, A. et al., "Plaque-Independent Disruption of Neural Circuits in Alzheimer's Disease Mouse Models" Proc. Natl. Acad. Sci. U.S.A., 1999 March 16; 96(6): 3228-3233 (**Exhibit 2**)

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

PNAS
Proceedings of the National Academy of Sciences of the United States of America

Proc. Natl. Acad. Sci. USA. 1999 March 16; 96 (6): 3228–3233
Neurobiology

Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models

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Abstract

Autosomal dominant forms of familial Alzheimer's disease (FAD) are associated with increased production of the amyloid β peptide, Aβ42, which is derived from the amyloid protein precursor (APP). In FAD, as well as in sporadic forms of the illness, Aβ peptides accumulate abnormally in the brain in the form of amyloid plaques. Here, we show that overexpression of FAD ($717_{V\square F}$)-mutant human APP in neurons of transgenic mice decreases the density of presynaptic terminals and neurons well before these mice develop amyloid plaques. Electrophysiological recordings from the hippocampus revealed prominent deficits in synaptic transmission, which also preceded amyloid deposition by several months. Although in young mice, functional and structural neuronal deficits were of similar magnitude, functional deficits became predominant with advancing age. Increased Aβ production in the context of decreased overall APP expression, achieved by addition of the Swedish FAD mutation to the APP transgene in a second line of mice, further increased synaptic transmission deficits in young APP mice without plaques. These results suggest a neurotoxic effect of Aβ that is independent of plaque

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Introduction

Alzheimer's disease (AD) is a progressive dementing illness in which the brain becomes littered with neuritic amyloid plaques. These plaques are associated with degenerating neuronal processes and consist primarily of fibrillar aggregates of the amyloid β peptide, Aβ. Aβ is derived from the amyloid protein precursor (APP), presumably via proteolytic cleavage of APP by β- and γ-secretases (1). The predominant forms of Aβ are 40 (Aβ40) or 42 (Aβ42) amino acids in length (2). Aβ42 and Aβ40 appear to be generated in different intracellular compartments, and Aβ42 has a greater propensity to self-aggregate into insoluble fibrils than Aβ40 (3, 4). Various point mutations in three distinct genes (APP, presenilin 1, presenilin 2) have been linked to autosomal dominant forms of familial AD (FAD). Notably, all of these mutations increase the production of Aβ42 (5).

Although Aβ has been shown to be neurotoxic in cell culture (6–8), a causal role for Aβ in widespread neuronal degeneration *in vivo* remains speculative. A particularly controversial question concerns whether Aβ-induced neurotoxicity requires deposition of aggregated Aβ into plaques (9–13). Transgenic mice in which full-length FAD-mutant APPs and Aβ are coexpressed at high levels develop typical neuritic amyloid plaques (14–17). However, loss of neurons so far has been identified in only one of these models (18) whereas two others showed no significant neuronal loss despite extensive cerebral Aβ deposition (19, 20). No electrophysiological studies have been reported in these models.

In the current study, we investigated in transgenic mice what early effects neuronal expression of full-length, FAD-mutant human APP has on the anatomy and physiology of the hippocampus, a central nervous system structure considered crucial for learning and memory. Our study demonstrates that the development of structural and functional neuronal deficits substantially precedes the formation of extracellular amyloid plaques and provides indirect evidence that Aβ, rather than APP itself, disrupts neuronal circuits in APP transgenic mice.

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MATERIALS AND METHODS

Transgenic Mouse Lines. The platelet-derived growth factor (PDGF)–APP_{Ind} transgene (14, 21) and the generation of PDGF-APP_{Ind} line H6 (22) have been described. The Swedish mutation was introduced into the PDGF-APP_{Ind} transgene by PCR primer modification, and the correctness of PCR-amplified regions was confirmed by sequencing essentially as described (21). Microinjection of the PDGF-$APP_{Sw, Ind}$ transgene into (C57BL/6 × DBA/2) F2 one-cell embryos, identification of transgenic founders by slot blot analysis of genomic DNA, and selection of the $APP_{Sw, Ind}$ expresser line J9 by RNase

protection assay (RPA) analysis were carried out according to previously described procedures (14, 21). Transgenic lines were maintained by crossing heterozygous transgenic mice with nontransgenic (C57BL/6 × DBA/2) F1 breeders. All transgenic mice were heterozygous with respect to the transgene. Nontransgenic littermates served as controls.

Mice were killed by decapitation under halothane anesthesia or by transcardial saline perfusion under anesthesia with chloral hydrate. Brains were removed rapidly and were dissected into regions to be snap-frozen immediately for later RNA and protein analyses, drop-fixed in phosphate-buffered 4% paraformaldehyde at 4°C for 24–72 h for neuropathological analysis, or used immediately for electrophysiological experiments.

RNA Analysis. RNA extractions and mRNA quantitations by solution hybridization RPA were performed as described (21) by using 10 μg of total RNA per sample in combination with the following ^{32}P-labeled antisense riboprobes [protected nucleotides (GenBank accession no.)]: human APP (hAPP) [nt2468–2657 (X06989) of hAPP fused via *Not*I linker with nt2532–2656 (M24914) of SV40] and actin [nt480–559 (X03672) of mouse β-actin].

Detection of APP and Aβ. Homogenization of snap-frozen hippocampi in guanidine buffer and ELISA quantitations of human full-length (FL) and α-secreted (α) APP, total Aβ, and Aβ1–42 were performed as described (23). For detection of Aβ deposits, vibratome sections were incubated overnight at 4°C with biotinylated mouse monoclonal antibody 3D6 (diluted to 5 μg/ml), which specifically recognizes $A\beta_{1-5}$ (22, 23). Binding of primary antibody was detected with the Elite kit from Vector Laboratories by using diaminobenzidine and H_2O_2 for development. Sections were counterstained with 1% hematoxyline and were examined with a Vanox light microscope (Olympus, New Hyde Park, NY). Four sections were analyzed per mouse.

Assessment of Neurodegeneration. To determine the integrity of presynaptic terminals and neuronal cell bodies, vibratome sections were incubated overnight with mAbs against synaptophysin (1 μg/ml; Boehringer Ingelheim) or microtubule-associated protein 2 (1 μg/ml, Boehringer Ingelheim), followed by incubation with fluorescein isothiocyanate-conjugated horse anti-mouse IgG (1:75, Vector Laboratories). Sections then were transferred to SuperFrost slides (Fisher Scientific) and were mounted under glass coverslips with an antifading media (Vector Laboratories). The sections were imaged with a laser scanning confocal microscope (MRC1024; Bio-Rad) as described (14, 15) at a magnification of 630×. The three-dimensional numerical densities (expressed as counts per cubic millimeter) of synaptophysin-immunoreactive presynaptic terminals and microtubule-associated protein 2 immunoreactive neuronal cell bodies in the CA1 and CA3 subfields of the hippocampus were determined by using a modification of the stereological "disector" (24). A confocal image of synaptic boutons (disector grid: 105.26 μm^2) or neurons (disector grid: 2546.19 μm^2) was obtained, and then a second image was captured at the same x and y coordinates but at a greater depth (0.9 μm for synapses and 2 μm for neurons). The two images

were superimposed, and the number of immunolabeled objects traversing both planes was counted. Twelve such disectors were spaced randomly through three serial hippocampal sections per mouse (avoiding overlap between disectors) and were analyzed. The mean counts obtained from 12 disectors per case were used for subsequent statistical analyses.

Electrophysiology. Hippocampal slice preparation and recording were performed as described (25). The artificial cerebrospinal fluid contained (in mM): 119 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 Mg_2SO_4, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, and 10 glucose. Experiments were performed in the presence of picrotoxin (0.1 mM). Whole-cell recording electrodes were filled with a solution containing (in mM): 122.5 Cs-gluconate, 11 EGTA, 10 CsCl, 10 Hepes, 8 NaCl, 10 glucose, 1 $CaCl_2$, 4 Mg-ATP, and 0.3 Na_3-GTP. Unless otherwise specified, cells were voltage-clamped at 70 mV.

Basal synaptic transmission was assayed by determining input–output relations from extracellular field potential recordings in the stratum radiatum of CA1; the input was the peak amplitude of the fiber volley, and the output was the initial slope of the excitatory postsynaptic potential (EPSP). Long-term potentiation (LTP) was induced with four tetani delivered 20 s apart, each at 100 Hz for 1 s.

Paired-pulse facilitation was elicited by using an interstimulus interval of 40 ms. The *N*-methyl-D-aspartate (NMDA)/α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) ratio was determined by holding cells at +50 mV. The peak amplitude of the average NMDA receptor-mediated excitatory postsynaptic current (EPSC) was divided by the peak amplitude of the average AMPA receptor-mediated EPSC recorded in the presence of the NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (D-APV) (50 μM), essentially as described (25).

In a separate series of experiments, we tested whether slice preparation itself could exacerbate excitotoxicity in transgenic slices and thereby lead to the observed deficits in basal synaptic transmission. Hippocampal slices were prepared from three 8- to 9-month-old APP_{Ind} mice either in the presence (n = 11) or absence (n = 9) of the glutamate receptor antagonist kynurenate (10 mM). Slopes of input–output relations were measured after removal of kynurenate. Pretreatment of slices with kynurenate did not prevent the impairment of input–output relations in transgenic slices ($P > 0.7$).

Statistical Analysis. For all experiments, mice and brain tissue samples were coded to blind investigators with respect to genotype. Unless indicated otherwise, data were expressed as mean ± SEM. Significance ($\alpha = 0.05$) was determined by Student's t test (pairwise comparisons), single-factor ANOVA followed by the Tukey-Kramer or Duncan's procedure (multiple comparisons), or the Pearson product–moment correlation coefficient t test (regression analyses).

RESULTS AND DISCUSSION

Age-Related Deposition of Aβ in Neuritic Plaques. In the first line of mice studied (H6) (22), the PDGF B chain promoter directs high-level neuronal expression of an alternatively spliced minigene encoding $717_{V\square F}$-mutant human APP695, APP751, and APP770 (21). Because the $717_{V\square F}$ substitution (APP770 numbering) has been linked to FAD in Indiana (26), hAPP carrying this mutation subsequently will be referred to as APP_{Ind}. High-level neuronal expression of the PDGF-APP_{Ind} fusion gene in another line of transgenic mice (line 109) has been shown to result in the development of AD-like neuropathology, including prominent amyloid plaques, dystrophic neurites, and gliosis (14, 15). Similar central nervous system alterations were subsequently also identified in transgenic models expressing FAD-mutant APPs from other promoters (16, 17).

Transgene expression levels in brains of APP_{Ind} mice from line H6 were similar to those of mice from line 109 (Fig. 1 *a* and *b*) (21, 23). Immunostaining with an hAPP-specific antibody (8E5) revealed widespread neuronal hAPP expression in brains of mice from line H6, with maximal levels found in the neocortex and hippocampus (data not shown). Hippocampal levels of hAPP and Aβ increased with age (Fig. 1 *b*). In addition, deposition of Aβ in the form of AD-like amyloid plaques was age-dependent (Fig. 1 *c* and *d*): Amyloid plaques were found in 45% (9 of 20) of mice 8–10 months of age whereas no amyloid plaques were found in mice 2–5 months of age ($n = 9$). We therefore studied hippocampal anatomy and physiology of mice at both of these ages to see whether amyloid plaques are necessary for any observed neuronal deficits to occur.

Decreased Density of Presynaptic Terminals and Neurons Precedes Plaque Formation. Losses of the presynaptic vesicle protein synaptophysin in the prefrontal cortex (27) and hippocampus (28) have been shown to correlate with cognitive decline in human AD cases. In addition, there is loss of hippocampal neurons in AD with the most prominent losses seen in CA1 (29). The densities of synaptophysin-immunoreactive presynaptic terminals and microtubule-associated protein 2-positive neurons in the CA1 region were 26–32% lower in 2- to 3-month-old APP_{Ind} mice than in nontransgenic controls (Fig. 2). Neuronal loss in the CA3 region became statistically significant only in older animals but, in some of these cases, was rather striking (Fig. 2 *d* and *f*). These findings are in contrast to the lack of neuronal loss in mice from line 109 (ref. 20; E.M., unpublished observations), which express the same PDGF-APP_{Ind} construct. Strain differences may explain this discrepancy. Line 109 was maintained on an outbred background (C57BL/6 × DBA/2 × Swiss–Webster) whereas line H6 was maintained on a hybrid background (C57BL/6 × DBA/2). It is well known that susceptibility to other types of neuronal injury (e.g., excitotoxin-induced neurodegeneration) also can vary widely across mouse strains (30). Of interest, loss of neurons in CA1 has recently also been observed in another transgenic line in which high-

level neuronal expression of FAD-mutant hAPP695 was directed by the Thy-1 promoter (18). To resolve the apparent discrepancies among different APP transgenic models will likely require concerted long-range efforts among different laboratories because all mouse lines will have to be backcrossed onto the same genetic background and analyzed side-by-side with the same methodologies.

Functional Decline Outstrips Neuropathological Alterations. Although histological identification of neuronal structures is informative, recent results caution against reliance on morphological information alone to make conclusions about the number of neuronal elements that are truly functional. Anatomical identification of presynaptic terminals can overestimate the number of functional synapses: presynaptically, there may not be active transmitter release (31–34), and postsynaptically, there may be an absence of receptors that are active at resting membrane potential (35, 36). Similarly, anatomical neuron counting may include neurons that are functionally removed from circuits (e.g., unable to generate action potentials). We therefore used electrophysiological techniques to assess whether there are functional changes in addition to the observed anatomical deficits, again at ages before and after amyloid plaque formation.

Extracellularly recorded EPSPs (field EPSPs) were used to assess the strength of basal synaptic transmission between hippocampal CA3 and CA1 cells. In 1- to 4-month-old APP_{Ind} mice, an 40% decrease in the slope of the input–output curve was observed (Fig. 3 *a*), indicating a significant impairment in synaptic transmission. This functional deficit is similar in magnitude to that observed anatomically in young mice (Fig. 2 *a*). However, by 8–10 months of age, a >80% deficit in basal synaptic transmission was observed (Fig. 3 *a* and *b*), suggesting a functional decline in great excess of morphological changes at that age.

The decrement in synaptic transmission is unlikely to be due to a decrease in the probability of transmitter release (p_r) because paired-pulse facilitation, which correlates inversely with the probability of transmitter release (37–39), remained unchanged (Fig. 3 *c*). Nor can this decrement be explained by a graded decrease in the responsiveness to transmitter at individual synapses, because the average amplitude of miniature EPSCs was similar in transgenic mice and nontransgenic controls (Fig. 3 *d*). Because neither the reliability nor the strength of individual synapses decreased, it is likely that a significant decrease in the number of functional synapses occurs between 3 and 8 months of age. This change is unlikely to be caused by the extracellular deposition of amyloid plaques because the magnitude of the functional deficit in 8- to 10-month-old APP_{Ind} mice did not correlate with the presence of plaques ($P > 0.5$, $n = 6$ mice; data not shown).

Analysis of Remaining Functional Synapses. Expression of APP_{Ind} thus appears to disconnect, both anatomically and functionally, neuronal subregions in the hippocampus. We next asked whether, among the functional

synapses that remain, there are any alterations in the ability to undergo plastic change. We therefore measured LTP in the CA1 region of APP_{Ind} mice but found no impairment (Fig. 4 *a* and *b*). At 30 min after induction, LTP was 167 ± 13% in APP_{Ind} mice ($n = 9$) and 163 ± 13% in nontransgenic controls ($n = 7$) ($P > 0.8$; age, 8–10 months). To ensure that the LTP in these mice was stable, in a subset of experiments LTP was monitored until 1 h after induction (Fig. 4 *b*); it averaged 199 ± 27% in 8-month-old APP_{Ind} mice ($n = 3$). In contrast, an impairment of LTP previously has been reported in transgenic mice in which a C-terminal fragment of hAPP was expressed (40), presumably in the cytoplasmic compartment of neurons, as opposed to the transmembrane localization of endogenous hAPP. The handling, trafficking, and signaling properties of this hAPP fragment are likely different from those of the full-length hAPP molecule and its natural cleavage products, which may explain the different results obtained in the two mouse models.

Another potential change at individual synapses is the proportion of synaptic transmission mediated by different receptor subtypes (41–44). The EPSC has two components generated by the NMDA and AMPA subtypes of glutamate receptors. We observed in APP_{Ind} mice an increased ratio of the NMDA versus AMPA components of the EPSC (Fig. 4 *c* and *d*), suggesting either an up-regulation of NMDA receptors or the inhibition/internalization of AMPA receptors at individual synapses. Because the AMPA receptor component of the miniature EPSCs did not decrease in amplitude in APP_{Ind} mice (Fig. 3 *d*), an up-regulation of NMDA receptors is more likely. Consistent with this interpretation, acute application of recombinant Aβ has been reported to selectively up-regulate NMDA receptor-mediated, but not AMPA receptor-mediated, synaptic transmission in hippocampal slices (45). Conceivably, Aβ-induced up-regulation of NMDA receptors could contribute to excitotoxicity and neuronal degeneration (46).

Increasing Aβ Production While Decreasing hAPP Expression Worsens Neuronal Deficits. In all AD models in which Aβ is expressed from the full-length precursor molecule, overexpression of Aβ is inseparably linked to overexpression of APP itself. Because APP could affect neuronal function through a number of different mechanisms (47–52), it is important to determine whether APP *per se* might be responsible for functional deficits observed in these models. We therefore generated a second mouse line in which Aβ is expressed at high levels in the context of relatively low levels of hAPP expression. This second mouse line ($APP_{Sw, Ind}$ line J9) was generated by introducing into the original APP_{Ind} transgene the "Swedish" mutation ($670_{K\square N}/671_{M\square L}$) (53), which has been shown to increase the generation of Aβ (54, 55). Mice from $APP_{Sw, Ind}$ line J9 had almost twice as much Aβ in their hippocampi as mice from APP_{Ind} line H6 but much lower hAPP levels (Fig. 5 *a–c*).

Because they are sensitive to both functional and anatomical changes, electrophysiological measures were used to compare 2- to 4-month-old mice

from lines H6 and J9. We reasoned that, if APP itself exerted the predominant deleterious effect in these models, mice from line J9 should display smaller deficits than mice from line H6 whereas the opposite would occur if Aβ were the main culprit. As shown in Fig. 5 *d*, the deficit in synaptic transmission in line J9 was almost twice as large as that in line H6. These findings are consistent with an insidious role for Aβ; however, to determine whether the neuronal deficits in these models are caused solely by Aβ, it will be necessary to develop compounds that selectively block Aβ production or activity without affecting other APP metabolites.

Although amyloid plaques were found in $APP_{Sw, Ind}$ mice from line J9 at 8–10 months of age, no amyloid plaques were detected in these mice at ages analyzed electrophysiologically (0 of 19 mice at 2–4 months of age). This finding underscores the fact that extracellular deposition of fibrillar Aβ is not required for the development of severe functional deficits in these models. If not extracellular deposits of fibrillar Aβ, what, then, is causing these impairments? Possibilities include neurotoxic effects induced by diffusible Aβ oligomers (8) or by intraneuronal accumulation of Aβ (4, 15, 56).

It is tempting to speculate that the disruption of neuronal connectivity we identified in the hippocampus of APP mice may relate to cognitive impairments seen in humans with AD. Although great caution must be applied when extrapolating from findings obtained in experimental models to complex human diseases, our results could provide a circuit-level explanation for the discrepancies observed between plaque load and functional deficits in humans with AD (11–13). They also suggest that inhibition of plaque formation alone may not prevent Aβ neurotoxicity *in vivo* and that inhibition of neuronal Aβ production may be required to achieve this therapeutic goal.

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ABBREVIATIONS

AD, Alzheimer's disease; APP, amyloid protein precursor; FAD, familial AD; PDGF, platelet-derived growth factor; RPA, RNase protection assay; FL, full-length; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; EPSC, excitatory postsynaptic current; hAPP, human APP; EPSP, excitatory postsynaptic potential; LTP, long-term potentiation; nt, nucleotides.

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Figures and Tables

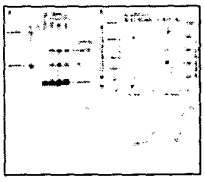
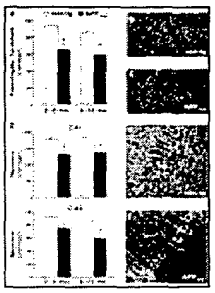


Figure 1. Expression levels of transgene products and age-related Aβ deposition in brains of APP_{Ind} mice.

Figure 2. Decreased density of presynaptic terminals and neurons in the hippocampus of APP_{Ind} mice (line H6).



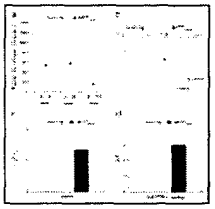


Figure 3. Severe impairment in synaptic transmission between hippocampal CA3 and CA1 cells in APP_{Ind} mice (line H6).

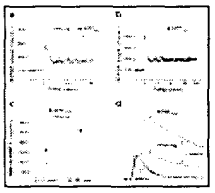


Figure 4. APP_{Ind} mice (line H6) showed normal LTP and an increase in the NMDA/AMPA ratio in CA1 cells.

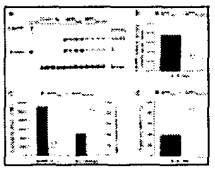


Figure 5. Increased Aβ levels exacerbate synaptic transmission deficits in the context of lower APP expression.